REMARKS

Claims 30-59 are pending. The Office finds that claims 30-33 are allowable and that claims 47-49 would be allowable if rewritten in independent form. Office Action, page 2. Claims 34-46 and 50-59 remain rejected under 35 U.S.C. §§ 102(e) and 103(a). Applicants address each new rejection below, according to its statutory origin.

Anticipation Rejection

Claims 34, 35, 38-40, 42-46, and 50-59 stand rejected under 35 U.S.C. § 102(e) in light of U.S. Patent 5,763,158 ("Bohannon"). Office Action, page 2. According to the Office, Bohannon teaches a method of detecting an analyte in a sample comprising the steps recited in independent claim 34. Office Action, pages 2 and 3. When referring to the "sample" recited in claim 34, the Office cites to the "ligand" discussed in Bohannon. Office Action, page 3. The Office contends that Bohannon teaches a first reagent comprising an ECL label at col. 4, lines 10-60. *Id.* The second reagent having an ECL quenching moiety as described in claim 34 is allegedly taught at col. 4, lines 35-46. *Id.* The Office specifically points to the apparent teaching of rhodamine as a quencher and notes that rhodamine contains a benzene moiety. *Id.* When discussing steps b-d of claim 34, the Office cites to col. 4, lines 15-21 for contacting an electrode and applying a potential to the electrode and to lines 30-35 for detecting a difference in ECL emissions. *Id.* at page 4. In the prior response, Applicants explained that Bohannon does not anticipate the rejected claims because this reference does not teach rhodamine as an ECL quencher. Based on the Office's reply to this argument, Applicants further develop this argument below in the interests of advancing prosecution. Applicants provide this additional explanation while addressing the Office's comments as well.

Bohannon does not teach rhodamine as an ECL quencher

Bohannon provides no structural information on an ECL quencher, instead defining the quencher in functional terms as a substance that absorbs light at 620 nm or destabilizes the ECL compound when in close proximity. Bohannon also appears to suggest that a quencher may operate by destabilizing a ruthenium compound. Col. 4, lines 24-28. Bohannon referred to rhodamine as a quencher of fluorescein, which is a fluorescent dye and not an ECL label.

The Office continues to employ an interpretation of Bohannon that contradicts the reference's own teaching. In column 4, Bohannon presents two different methods of detection. First, it presents a method of detection using ECL. Second, it presents an alternative method of using fluorescence. In citing to Bohannon, the Office is not accounting for the fact that these two paragraphs discuss two different methods—instead the Office is improperly combining the teachings on ECL and fluorescence without accounting for the differences in these methods.

Prior to discussing the specifics of the rejection and the differences between the fluorescence and ECL teachings, Applicants present the relevant paragraphs from Bohannon. The first quoted paragraph presents Bohannon's teachings on ECL.

> For binding sites containing a mAb bound to the ligand, the detection of antibody-antigen reactions may also be achieved using reporter molecules other than enzymes as the enzyme portion of the ligand-enzyme complex. For example, a modification of the recently developed **electrochemiluminescence (ECL) technology** (available from IGEN, Gaithersburg, Md.) may be used in the present invention, by replacing the enzyme portion of the ligand-enzyme complex with a **ruthenium-labeled molecule**. The resulting complex will be referred to as a ligand-reporter complex. Applying a low voltage to an **electrode** positioned near the binding site oxidizes the ruthenium label and its

> substrate, tripropylamine (TPA), to form strong oxidant and reductant compounds, respectively, which then react to form an excited state of the ruthenium label which decays to its starting ground state while releasing a photon at 620 nm. (See, e.g., Michael Carlowicz, "Electrochemiluminescence Could Spark an Assay Revolution", Clin. Lab. News, Vol. 21, p. 1-2 (August 1995), herein incorporated by reference.) The photon emissions are detected using a photo multiplier tube (PMT). The mAb is labeled with a quencher molecule capable of reducing detectable reporter activity, i.e., photon emissions, either by adsorbing light at 620 nm or by destabilizing the ruthenium compound when the quencher is located a short distance from the ligand-reporter complex. Thus, when the mAb is bound to the ligand-reporter complex, the amount of detectable emissions from the binding site is reduced, or suppressed, by the quencher molecule. Upon displacement from the complex of the mAb by a competing antigen or antibody, the amount of detectable emissions, or signal, increases.

Col. 4, lines 5-35 (emphasis added). The second quoted paragraph presents an ***alternative*** to ECL, specifically it presents a fluorescence method of detection, providing information on how to conduct the fluorescence method. As we will discuss below, it is important to note that this presents a different method, one using fluorescence, not ECL.

> Those skilled in the art will recognize that **other reporter-quencher dye systems may be used** in a manner similar to the above-described modification of ECL, wherein the reporter portion of the ligand-reporter complex comprises a molecule labeled with the **reporter dye** and the mAb is labeled with a quencher dye. For example, the reporter dye may be **fluorescein** and the quencher dye may be rhodamine. In embodiments where the signal is produced by a reporter molecule other than a classical enzyme, the term "enzyme's active site" means the reporter compound and "enzymatic activity" means detection of **fluorescence**.

Col. 4, lines 36-46 (emphasis added).

In the discussion of the ECL method, there is no specific teaching of any quenchers and Bohannon does not teach that rhodamine is an ECL quencher. If

anything, Bohannon provides only a functional definition of an ECL quencher as discussed above. Given a purely functional definition, the skilled artisan would not specifically envision an ECL quenching moiety comprising at least one benzene moiety as recited in claim 34. Moreover, when describing ECL, Bohannon uses the term "label" or "reporter." And when discussing an ECL quencher, Bohannon uses the term "quencher molecule." Col. 4, line 25. In contrast, Bohannon describes rhodamine as a "quencher dye" that could be used with a "reporter dye" such as fluorescein. *Id.* at lines 41 and 42. If Bohannon had contemplated rhodamine as an ECL quencher, this reference would have discussed rhodamine in the paragraph on ECL quenchers and used the same terminology to refer to rhodamine (i.e., called rhodamine a quencher molecule rather than a quencher dye).

Instead, Bohannon discusses rhodamine in only in the second quoted section—the one focusing on the alternative fluorescence method. Therefore, rhodamine is presented only in the context of a fluorescent label, fluorescein and is conspicuously absent from the discussion of the ECL method. Indeed, Bohannon instructs that "other reporter-quencher dye systems may be used in a manner similar to the above-described modification of ECL." Col. 4, lines 36-38. Thus, by referring to rhodamine in this paragraph, Bohannon clearly contemplates its use in other reporter systems rather than with ECL. The last sentence of the paragraph beginning at line 36 refers to the detection systems discussed in that paragraph as involving detection of fluorescence. Thus, by discussing rhodamine as a quencher dye in this paragraph, Bohannon at best may contemplate rhodamine as a fluorescence quencher, not an ECL quencher.

When describing fluorescent dyes and quencher dyes, Bohannon discusses them in pairs. See col, 4, paragraph beginning at line 36. According to Bohannon, the fluorescent dye could be in a reporter complex while the quencher dye is on a monoclonal antibody. Directly after this apparent teaching, Bohannon then suggests that "the reporter dye may be fluorescein and the quencher dye may be rhodamine." Lines 41-42. Thus, Bohannon discusses rhodamine as one part of an exemplary pair of a fluorescent dye (fluorescein) and a matching quencher dye. Given the context in which Bohannon discusses rhodamine as a quencher, the Office's conclusion that this Bohannon teaches rhodamine as an ECL quencher is unsupported by the reference.

In addition, the Office now suggests that a teaching that a quencher can quench one source of photon emissions is a direct teaching that the quencher can quench any source within its wavelength range. Office Action, page 8. Applicants disagree. As the specification explains, fluorescence and electrochemiluminescence occur via different mechanisms. See paragraphs [004] and [005]. Fluorescence results when photoluminescence is the result of a spin-allowed transition (e.g., a single-singlet transition, triplet-triplet transition). Electrochemiluminescence pertains to the emission of photons from an electronically excited chemical species which has been generated electrochemically. Thus, the ability of a quencher to quench a fluorescent label does not necessarily teach whether that quencher will also quench a label that emits a signal via a different mechanism.

Indeed, the specification also provides several possible ways in which ECL quenchers could function including transferring an electron to the quencher, absorbing a photon emitted by the ECL label, electrochemically converting to an electro-oxidation or

electro-reduction product, and acting as a free radical scavenger to intercepts one or more species involved in the ECL reaction. See specification at paragraph [103]. Bohannon merely suggests that rhodamine "may be" a fluorescence quencher and does not provide any guidance on how rhodamine might quench a fluorescent label. See col. 4, lines 41 and 42. There are also several potential mechanisms underlying fluorescence quenching including collisional or dynamic quenching, static quenching, quenching by energy transfer, and charge transfer reactions. Without knowing how rhodamine quenches fluorescence, the artisan cannot know if rhodamine could be equally applied to an ECL label. Thus, the Office's premise that a teaching that a quencher can quench one source of photon emissions is a direct teaching that the quencher can quench any source within its wavelength range is not accurate when several potential mechanisms of quenching are involved. Moreover, the Office's premise is not taught by Bohannon and instead has been read into the reference.

Obviousness Rejection

The Office maintains its rejection of claims 36, 37, and 41 as obvious in light of Bohannon and Kuzmin et al., *J. Photochem. Photobiol. A: Chem* 87:43-54 (1995). Office Action, page 5. The Office uses Bohannon as described above, acknowledging that this reference does not teach quinones or phenols as quenchers or an ECL label comprising osmium. *Id.* The Office turns to Kuzmin for allegedly teaching quinones as quenchers and asserts that quinones encompass phenols. Office Action, page 6. The Office also notes that Kuzmin does not provide any examples of assays using an osmium label, but contends that Kuzmin teaches ruthenium and osmium as equivalents. *Id.* In light of these alleged teachings, the Office concludes that it would obvious to

substitute quinone and phenolic quenchers into Bohannon's assay and to use osmium as an ECL label in Bohannon's assay. The Office also now contends that Applicants have considered the limitations of Bohannon and Kuzmin separately rather than addressing their combination. *Id.* at page 10. Applicants disagree.

In the prior response, Applicants provided absorption spectra for benzene, phenol, and benzoquinone, demonstrating that none of these compounds absorb light at 620 nm. As discussed above, Bohannon clearly defines an ECL quencher as a substance that absorbs light at 620 nm. Applying the teachings of Bohannon and Kuzmin together, even if Kuzmin did teach quinone and phenolic compounds as quenchers, the skilled artisan would not be motivated to combine these two references. The physical characteristics of three examples of substances that qualify as the quinone and phenolic compounds allegedly taught in Kuzmin, namely benzene, phenol, and benzoquinone, do not fit the functional definition of an ECL quencher provided in Bohannon. In light of this conflict resulting from the combination of both references, the skilled artisan would not be motivated to combine Bohannon and Kuzmin by substituting quinone and phenolic compounds as ECL quenchers in Bohannon's assay. Applicants note that the Office has not addressed this argument in the current Office Action.

Regarding claim 41, the Office continues to assert that Kuzmin teaches osmium and ruthenium as equivalents, using this alleged teaching as a basis for suggesting that it would be obvious to use osmium in the assay of Bohannon. Office Action, page 7. It is the Office's initial burden to present a *prima facie* case of obviousness. See M.P.E.P. § 706.02(j). To date, the only support the Office provides is a referral to a Court of Customs and Patent Appeals case, *In re Fout*, which apparently instructs that for

equivalence to support an obviousness rejection, the equivalence must be recognized in the art. Office Action, page 10. In connection with this case, the Office cites Kuzmin at page 51, right column, paragraph 4 and simply asserts that Kuzmin teaches that osmium and ruthenium are equivalents. The Office provides absolutely no explanation as to why this it believes that this citation to Kuzmin teaches the skilled artisan that ruthenium and osmium are equivalents. In the absence of such an explanation, the Office has not met its burden of establishing the foundation for a *prima facie* case of obviousness. Indeed, the M.P.E.P. further instructs that it is important for the Office to properly communicate the basis for a rejection so that the issues can be identified early and the Applicant can be given fair opportunity to reply. See M.P.E.P. § 706.02(j).

In contrast, Applicants have explained why this citation does not teach the equivalence of ruthenium and osmium as ECL labels to the skilled artisan. This section of Kuzmin discusses an apparent inconsistency in the data. Based on ΔG values, it appeared that the sample reactants were in a polar microenvironment. But the spectral properties of RuL_6^{2+} suggested a low polarity environment. Kuzmin provides an explanation for this discrepancy, discussing the localized effect water molecules might have on RuL_6^{2+}. In this discussion, Kuzmin notes a study looking at the isotope effect on the lifetimes of osmium (Os) and ruthenium (Ru) complexes and micellar solutions showed that about 30% of the ligand surface of RuL_6^{2+} was accessible to water molecules. Assuming that Kuzmin's description of this study is accurate, this study addresses the behavior of Os and Ru compounds in micellar environments. This study does not, however, address the ability of Os compounds to act as ECL labels as recited in claim 41 let alone show an equivalence between the two metals in the context of an

ECL label. Thus, the motivation to use Os instead of Ru in Bohannon's assay does not exist. Because the combination of Bohannon and Kuzmin does not render the rejected claims obvious, Applicants request that the Office withdraw this rejection.

Status of the Claims

Applicants noted in the last response that the Office discussed claims 43-59 as allowable subject matter. The Office continues to do so in the current Office Action. Applicants await the Office's clarification as to whether claims 43-59 are allowable, given that claims 43-46 and 50-59 remain rejected.

Conclusions

In view of the foregoing remarks, Applicants respectfully request reconsideration and reexamination of this application and the timely allowance of pending claims 30-59. Applicants representatives also request an interview in the interests of advancing prosecution.

Please also grant any extensions of time required to enter this response and charge any additional required fees to Deposit Account No. 06-0916.

Respectfully submitted,

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